

# Acetone and Isoprene Concentrations in Exhaled Breath in Healthy Subjects

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We measured the concentrations of acetone and isoprene in the exhaled breath from students to evaluate their lifestyles for the annual medical checkup at our university. 451 students were examined for both gases simultaneously by a gas analysis device, Biogas Acetone Analyzer (BAS-2000). The average concentrations of acetone and isoprene in the breath were  $0.53 \pm 0.45$  ppm and  $0.065 \pm 0.058$  ppm, respectively, and the frequency of the abnormal high levels by judging the cut-off values of 1.5 and 0.20 ppm were 3.5% and 2.7% of them, respectively. Acetone concentration in the breath was higher in students having no breakfast in the day of the medical checkup than those having breakfast. Furthermore, acetone concentration was significantly correlated inversely with body fat percentage in students who took the medical checkup in the morning and significantly higher in students with body mass index (BMI) under 25 than obese students with BMI over 25. On the other hand, isoprene concentration was higher in male students than female students, and also higher in smokers than nonsmokers. However, isoprene level was not affected by ingestion of breakfast in the day. Acetone and isoprene concentrations were correlated positively with each other, although the relationship was not affected by having breakfast. The results suggest that the measurement of acetone and isoprene concentrations in the breath is useful for evaluating lifestyles such as lack of breakfast, smoking and obesity in students.

**Key words**—gas analysis, acetone and isoprene in the breath, carbon monoxide, student lifestyle, medical checkup

## INTRODUCTION

Gas analysis to measure the concentration of volatile organic compounds (VOCs) in the exhaled breath is a low invasive method in order to obtain information on the living body.<sup>1)</sup> The previous study<sup>2)</sup> indicated a good correlation between concentrations of  $\beta$ -hydroxy-butyrate or acetoacetate in the blood and concentrations of acetone ( $\text{CH}_3\text{COCH}_3$ ) in the breath, and thus many reports<sup>3)</sup> suggested that acetone determinations in the breath must be an indicator for ketoacidosis. Acetone is an indicator for metabolizing fat accumulated in the abdominal cavity into the energy due to its  $\beta$ -oxidation, and thus acetone levels in the breath may be higher due to the progress of body weight loss by fasting or calories-limited diet (negative calories balance).<sup>4)</sup> Therefore, repeated measurements of acetone in the breath are useful for accurately monitoring effective loss of body weight or body fat mass, and thus can also be used as a motive for continuing dietary therapy of obesity.<sup>5)</sup> Determination of isoprene [ $\text{CH}_2\text{CHC}(\text{CH}_3)\text{CH}_2$ ] concentration in the breath is interested in knowing mainly the daily approximate amounts of cholesterol biosynthesized and effectiveness of drug therapy for hypercholesterolemia.<sup>6,7)</sup>

Recently, we have developed a new gas analysis device (Biogas Acetone Analyzer, BAS-2000)<sup>8)</sup> for determining the concentrations of acetone and isoprene. In this opportunity, we determined concentrations of acetone produced during  $\beta$ -oxidation of fatty acids and isoprene formed from dimethylallyl pyrophosphate during cholesterol biosynthesis<sup>9)</sup> to know lifestyles in the students such as lack of breakfast, smoking, being on a diet and obesity. Particularly, acetone concentration in the breath may be useful for monitoring effective progress of weight-loss during dietary therapy in obese patients.<sup>5)</sup> Thus, the present study was performed as a fundamental research to simultaneously determine two gases in the breath on 451 students at the time of the annual medical checkup at our university.

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## MATERIALS AND METHODS

**Medical Checkup of Students**— We performed gas analysis at the chance of the annual medical checkup of students at our university between 28 and 30 March 2007. During this period, we took samples for measuring acetone and isoprene concentrations in the end tidal air (alveolar air) of the exhaled breath of 451 students by using a gas analysis device Biogas Acetone Analyzer, BAS-2000. 336 students took the medical checkup in the morning (8:30–12:00) and 115 students in the afternoon (13:30–16:00). At the same time, we also measured carbon monoxide (CO) gas concentrations as an indicator of smoking by using a CO gas monitor (EC-50: ToxCO, New Micro Smokerlyzer, Bedford Instruments, Kent, U.K.). Body fat percentage was measured by using an impedance method (TANITA TBF-410, TANITA, Co., Inc., Tokyo, Japan). Before measurement of exhalation, the students filled out a questionnaire which was used for self-evaluation at the time of the analysis. Informed consent was obtained from all students after we had thoroughly explained the objectives, methods and other relevant details of the study. This research has been approved by the Ethics Committee of our university (No. 018).

**Exhaling Sampling**— Each student breathed in lightly and then waited 15 sec before exhaling. A breath sample was taken at the last moment of exhalation (end tidal volume), with each student exhaling between 100 and 200 ml into a breath-sampling bag manufactured by Otsuka Pharmaceutical Co., Ltd. (Osaka, Japan). The inside of bags was thoroughly washed at 60°C overnight 3 times by inflating with pure synthetic air (G1 grade: impurity less than 0.1 ppm) free from VOCs to exclude some contaminated gases generated from the inside surface of bags. As previously reported by us,<sup>10,11)</sup> measurement of concentration of CO in the breath was conducted by directly breathing into a CO gas monitor and the concentration was immediately indicated in ppm. The principal of measurement was based on the controlled-potential electrolysis method using an electrochemical sensor.<sup>11)</sup>

**Measurement of Exhaled Gas**— 2.5 ml of exhaled gas was taken with a syringe and introduced into Biogas Acetone Analyzer, which makes it possible to analyze acetone and isoprene for 3 min 12 sec and for 3 min 36 sec per sample, respectively. All the analyses were performed two to five days after the exhalation was sampled. The analysis device

used is a gas chromatograph equipped with a high-sensitivity semiconductor gas detector. The lower limit for detection of acetone and isoprene gases is 0.1 ppm and 0.001 ppm, respectively. Its reproducibility is  $\pm 1\%$  and its linearity reaches a level of 5 ppm. We used pure synthetic air as the carrier gas and performed calibration on every 50 samples using mixed gas of 5 ppm concentration for acetone and isoprene.

**Statistic Analysis**— Statistic analysis was performed by using SPSS 13.0J (SPSS Japan Inc., Tokyo, Japan). The results obtained are expressed in terms of the mean value  $\pm$  the standard deviation (S.D.), and the significant difference is verified by means of Student *t*-test, Chi-square test, Fisher's direct probability method and Spearman's correlation coefficient. We estimated the significant difference when *p* value is less than 0.05.

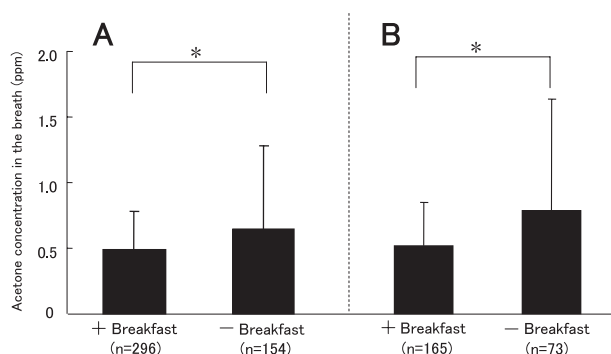
## RESULTS

### Acetone

Among the 451 students including 154 males and 297 females who took part in the test, the numbers of students and their distributions of each category for acetone and isoprene concentrations in the breath were shown in Table 1. The average concentration of acetone was  $0.53 \pm 0.45$  ppm, and no significant difference between male ( $0.50 \pm 0.33$  ppm) and female ( $0.55 \pm 0.49$  ppm) observed. The frequency of the students with abnormal (higher) levels obtained by using the cut-off values over 1.5 ppm as a reference level by judging from mean + 2 S.D. was 0.9% in males and 2.6% in females; the latter being non-significantly higher than the former ( $p < 0.06$ ). The acetone concentration in female students ( $n = 238$ ,  $0.57 \pm 0.53$  ppm), who took the medical checkup in the morning, were insignificantly higher than those in male students ( $n = 98$ ,  $0.48 \pm 0.31$  ppm,  $p = 0.06$ ). They were divided into two groups who had breakfast or not in the day of the medical checkup in accordance with "yes" or "no" given by students in the questionnaire. The concentration of acetone in the latter students without breakfast ( $0.65 \pm 0.63$  ppm) showed significantly higher than the former with breakfast ( $0.49 \pm 0.29$  ppm,  $p < 0.01$ ) (Fig. 1A). Moreover, when the students were restricted to female students who took the medical checkup in the morning, students without breakfast showed significantly higher levels of acetone in

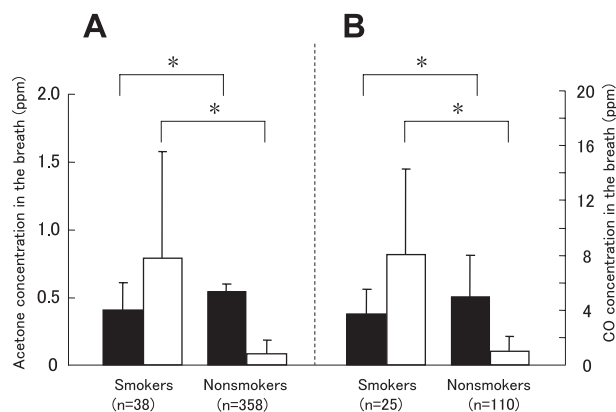
**Table 1.** Results of Measurement of Concentrations of Acetone and Isoprene Gases in the Exhaled Breath (According to Gender, in %)

Category	(ppm)	Total (%)	Male (%)	Female (%)
Acetone				
1	Under 0.5	297 (65.9)	104 (23.1)	193 (42.8)
2	0.5–under 1.0	115 (25.5)	40 (8.9)	75 (16.6)
3	1.0–under 1.5	23 (5.1)	6 (1.3)	17 (3.8)
4	1.5–under 3.0	14 (3.1)	4 (0.9)	10 (2.2)
5	Over 3.0	2 (0.4)	0 (0)	2 (0.4)
Isoprene				
1	Under 0.1	331 (73.4)	92 (20.4)	239 (53.0)
2	0.1–under 0.2	108 (23.9)	55 (12.2)	53 (11.8)
3	0.2–under 0.3	9 (2.0)	6 (1.3)	3 (0.7)
4	Over 0.3	3 (0.7)	1 (0.2)	2 (0.4)
Total		451 (100)	154 (34.1)	297 (65.9)

**Fig. 1.** Effect of Breakfast Ingestion on Acetone Concentrations in the Breath

A. Acetone concentrations in the breath in all the students with and without breakfast in the day for the medical checkup. 450 cases except one case who did not answer on breakfast in the questionnaire. Number of students is different from No. in Table 1 and the following figures, because some students did not answer in the questionnaire. \* $p < 0.01$ . B. Acetone concentrations in the breath in female students with and without breakfast who took the medical checkup in the morning. \* $p < 0.01$ .

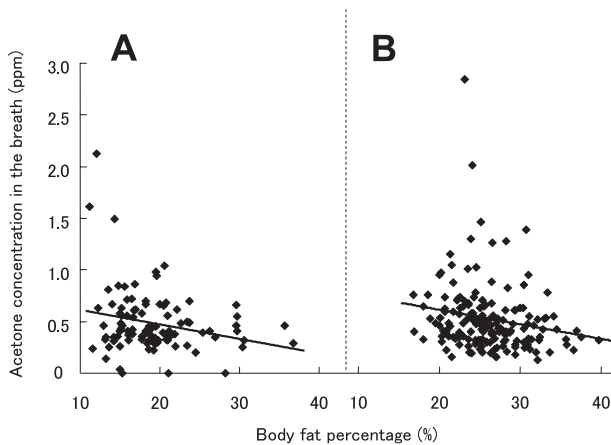
the breath than students with breakfast ( $0.74 \pm 0.80$  vs.  $0.49 \pm 0.31$  ppm,  $p < 0.01$ , Fig. 1B). Same analysis with male student showed no significant difference ( $0.50 \pm 0.39$  vs.  $0.46 \pm 0.17$  ppm). Furthermore, when the students were divided into smokers and nonsmokers, smokers showed significantly lower breath acetone concentrations than nonsmokers irrespective of the whole students ( $0.40 \pm 0.20$  vs.  $0.54 \pm 0.06$  ppm,  $p < 0.01$ , Fig. 2A) and male students alone ( $0.38 \pm 0.18$  vs.  $0.50 \pm 0.31$  ppm,  $p < 0.01$ , Fig. 2B). Smokers showed 10-fold higher CO concentrations in the breath as compared with nonsmokers, confirming that they are smokers (Fig. 2A and 2B). However, female nonsmokers ( $n = 201$ ), who took the medical checkup in the

**Fig. 2.** Effect of Smoking Habits on Acetone Concentrations in the Breath

A. Acetone (■) and CO (□) concentrations in the breath determined in the smokers or nonsmokers ( $n = 396$ ). \* $p < 0.01$ . B. Acetone (■) and CO (□) concentrations in the breath determined in the male smokers or nonsmokers ( $n = 135$ ). \* $p < 0.01$ .

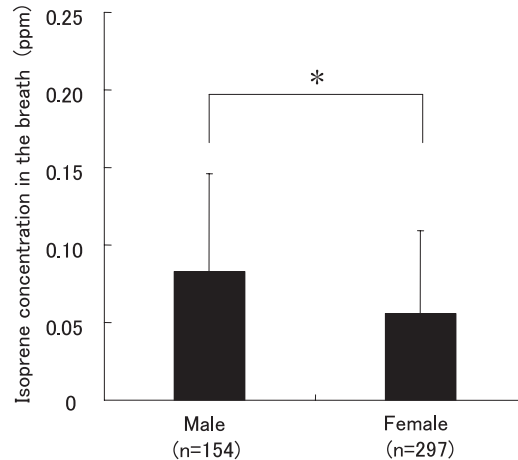
morning, showed  $0.69 \pm 0.75$  ppm in those without breakfast and  $0.49 \pm 0.35$  ppm in those with breakfast; the former being higher ( $p < 0.02$ ). This observation suggests that higher levels of breath acetone in nonsmokers must be due to lack of breakfast in female students without smoking.

A significant correlation between acetone concentration in the breath and body fat percentage was observed in male and female students who took the medical checkup in the morning ( $p < 0.05$ , Fig. 3A and 3B), but a correlation between acetone and lean body mass was not observed. Furthermore, acetone levels in the breath were higher in students with body mass index (BMI) under 25 than obese students with BMI over 25 ( $0.54 \pm 0.45$  vs.  $0.37 \pm 0.18$  ppm,  $p < 0.03$ , Fig. 4). Male students who took the medical checkup in the morning

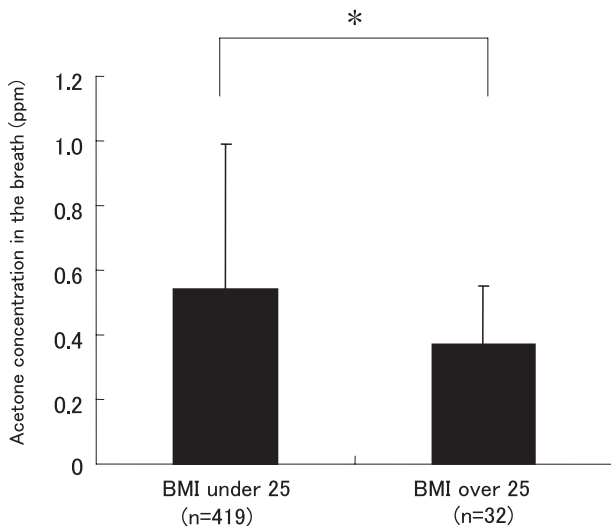


**Fig. 3.** Significant Correlation of Acetone Concentrations in the Breath and Body Fat Percentage in Male and Female Students Who Took the Medical Checkup in the Morning

A. Male students (all,  $n = 98$ ),  $y = -0.0132x + 0.7354$ ,  $p < 0.05$ .  
 B. Female students with breakfast ( $n = 165$ ),  $y = -0.0134x + 0.8424$ ,  $p < 0.05$ .



**Fig. 5.** Gender Difference of Isoprene Concentrations in the Breath  
 All the students ( $n = 451$ ).  $*p < 0.01$ .



**Fig. 4.** Acetone Concentrations in the Breath in Students with BMI under 25 or BMI over 25  
 $*p < 0.03$ .

showed higher levels in those with BMI under 25 ( $n = 85$ ,  $0.50 \pm 0.32$  ppm) than those with BMI over 25 ( $n = 13$ ,  $0.36 \pm 0.15$  ppm,  $p < 0.02$ ). Similarly, female students who took the medical checkup in the morning also showed the higher levels in cases with BMI under 25 ( $n = 224$ ,  $0.58 \pm 0.54$  ppm) than those with BMI over 25 ( $n = 14$ ,  $0.33 \pm 0.13$  ppm) ( $p < 0.01$ ). Further, the data expectedly confirmed a positive correlation between body fat percentage and BMI in all the students and a negative between body fat percentage and lean body mass (data not

shown).

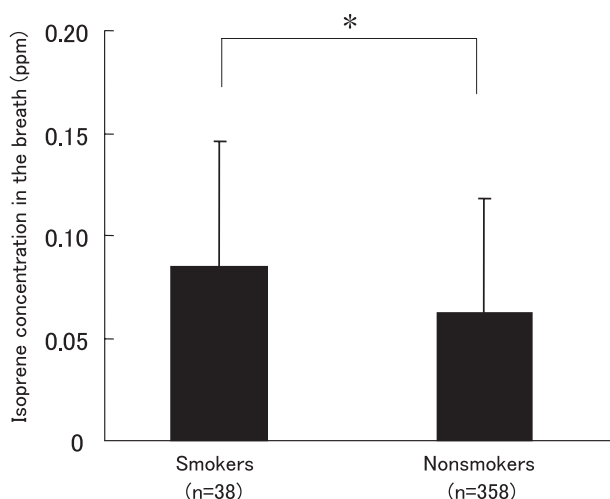
We also performed a comparison of the concentration of acetone in the breath in accordance with the replies in the questionnaire. However, there was no clear correlation with drinking habits, alcoholic beverages in the preceding day, daily physical activity, energetic physical activity, length of sleeping, and fatigue or stress state.

### Isoprene

Student numbers and their distribution of each Category were shown in Table 1. The average level of isoprene was  $0.065 \pm 0.058$  ppm, and the levels in male ( $0.083 \pm 0.063$  ppm) were significantly higher than in female ( $0.056 \pm 0.053$  ppm,  $p < 0.01$ , Fig. 5). The frequency of the students with showing abnormal (higher) levels (the cut-off levels: over 0.20 ppm) was 1.5% in male and 1.1% in female ( $p < 0.04$ ), respectively. The concentrations were not different between students who took the medical checkup in the morning and afternoon. The isoprene levels were higher in smokers ( $n = 38$ ,  $0.09 \pm 0.06$  ppm) than in nonsmokers ( $n = 364$ ,  $0.06 \pm 0.06$  ppm,  $p < 0.04$ , Fig. 6). Male smokers with below 30 cigarettes/day ( $n = 25$ ,  $0.102 \pm 0.063$  ppm) were higher than male nonsmokers ( $n = 110$ ,  $0.076 \pm 0.058$  ppm,  $p < 0.05$ ), but such differences were not observed in female smokers with below 10 cigarettes/day ( $n = 13$ ,  $0.050 \pm 0.041$  ppm) and nonsmokers ( $n = 248$ ,  $0.056 \pm 0.053$  ppm). However, significant correlation between breath CO and isoprene concentrations could not be observed ( $y = 0.002x + 0.0798$ ,  $p = 0.135$ ). The isoprene levels in the breath were

not related to the following lifestyle in accordance with the reply in the questionnaire such as their having a breakfast, a family history of hyperlipidemia, different anthropometric indexes (body fat percentage, BMI, lean body mass), duration of sleep, stress and fatigue conditions, ingestion of medicines or dietary supplements, and daily habits with physical activity or meal regularity.

The significant negative correlation between acetone and isoprene concentrations in the breath was observed in the all students ( $p < 0.01$ , Fig. 7), and the similar results were observed even by restricting to nonsmokers ( $p < 0.01$ ) or female students irrespective of having breakfast, who took the medical checkup in the morning ( $p < 0.01$ ).



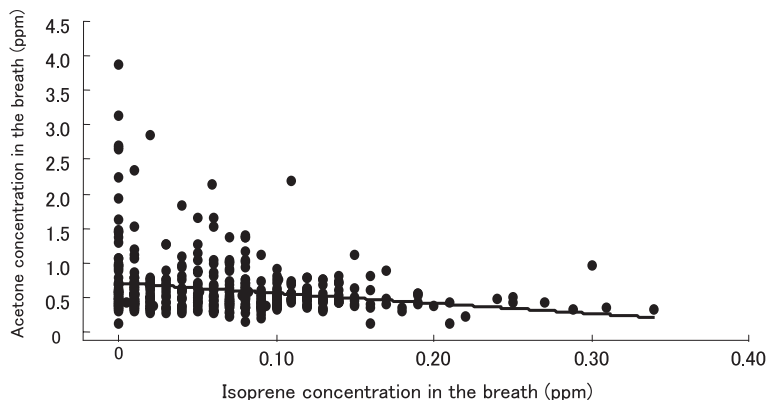
**Fig. 6.** Isoprene Concentrations in the Breath in Smokers and Nonsmokers

All the students ( $n = 396$ ) whose acetone and CO concentrations were simultaneously determined in the breath. CO concentrations were omitted because of the same data shown in Fig. 2. \* $p < 0.04$ .

## DISCUSSION

The interesting and new results obtained from the present study are described as follows. Acetone concentration in the breath is related to the ingestion of breakfast before breath sampling, and its levels in female students are significantly higher in those without breakfast than with breakfast. Furthermore, acetone concentrations showed a negative correlation with body fat percentage and higher in students with BMI under 25 than obese students with BMI over 25. Frequency of students without breakfast in the medical checkup day and with BMI over 25 was 42.9 and 11.6% in male and 33.0 and 5.7% in female, respectively. Both frequencies were significantly higher in the former than in the latter. Acetone concentration in the breath showed no gender difference probably by the fact that two factors such as breakfast and obesity are related to each other. The previous study showed that obese cases showed lower concentrations of acetone in the breath than in nonobese cases<sup>12)</sup> and ketosis occurred more slowly in obese cases than nonobese cases.<sup>4)</sup>

On the other hand, isoprene concentrations in the breath were higher in male students, suggesting that smoking must be related to the higher levels as a background. The previous report showed that acetone and isoprene levels in the breath did not elevate in 50 smokers (more than 20 cigarettes/day for longer than 2 years<sup>13)</sup>), although concentrations were transiently increased shortly after smoking. The degree of smoking was approximately estimated by measuring CO concentrations in the breath and both indexes are parallel related (for example: 20 cigarettes/day = 20 ppm).<sup>14)</sup> However, the present study did not show a direct correlation



**Fig. 7.** Significant Correlation of Acetone and Isoprene Concentrations in the Breath  
All the students ( $n = 451$ ).  $y = -1.0822x + 0.6063$ .  $p < 0.01$ .

between isoprene levels and CO concentrations in the breath. The reason may be the fact that there are few severe smokers as the subjects of the present study and thus there is a great variation of CO concentrations prior to CO measurement in the day by the fact how many cigarettes were smoked after getting up.

Estimated amounts of isoprene and acetone produced by smoking one cigarette are calculated to be similar, and its blood half-life was estimated to be 4–7 min in the former (exposure experiment in rats and mouse<sup>15)</sup>) and 17–24 hr in the latter (human).<sup>16)</sup> These data can not explain why only isoprene concentrations in the breath were correlated with smoking but not acetone. The other report suggested that acetone levels were not related to smoking and isoprene was not affected by gender, meal ingestion and fasting.<sup>17)</sup>

Further study will be needed for confirming the cut-off values of acetone and isoprene, evaluating the effect of smoking in the levels of both gases in the breath. The concentrations of acetone and isoprene are an indicator for intrinsic total amounts of both gases produced in the body, and thus it is necessary to repeatedly determine gas concentrations at many points of time (diurnal variation) as reflecting a total amounts produced in the body.<sup>18)</sup> Development of low invasive methods for clinical testing in order to obtain information on the living body is anticipated in the future. One of these methods may well involve breath gas. Two of the authors (A. W. and H. O.), both of whom are on the staff of Japanese Society of Clinical Biochemistry on Biogas Analysis, succeeded in developing Biogas Acetone Analyzer (BAS-2000) for use in investing people's lifestyles. In the future, this new machine will be widely used for preventing various diseases such as metabolic syndrome.<sup>19)</sup>

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